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Dimethylsulfoniopropionate (DMSP) and its catabolite dimethyl sulfide (DMS) are key marine nutrients[1,2], with roles in global sulfur cycling[2], atmospheric chemistry[3], signalling[4,5] and, potentially, climate regulation[6,7]. DMSP production was previously thought to be an oxic and photic process, mainly confined to the surface oceans.

However, here we show that DMSP concentrations and DMSP/DMS synthesis rates were higher in surface marine sediment from e.g., saltmarsh ponds, estuaries and the deep ocean than in the overlying seawater. A quarter of bacterial strains isolated from saltmarsh sediment produced DMSP (up to 73 mM), and previously unknown DMSP-producers were identified. Most DMSP-producing isolates contained *dsyB*[8], but some alphaproteobacteria, gammaproteobacteria and actinobacteria utilised a methionine methylation pathway independent of DsyB, previously only associated with higher plants. These bacteria contained a methionine methyltransferase '*mmtN*' gene - a marker for bacterial DMSP synthesis via this pathway. DMSP-producing bacteria and their *dsyB* and/or *mmtN* transcripts were present in all tested seawater samples and *Tara* Oceans bacterioplankton datasets, but were far more abundant in marine surface sediment. Approximately 10^8 bacteria per gram of surface marine sediment are predicted to produce DMSP, and their contribution to this process should be included in future models of global DMSP production. We propose that coastal and marine sediments, which cover a large part of the Earth's surface, are environments with high DMSP and DMS productivity, and that bacteria are important producers within them.

Approximately eight billion tonnes of DMSP is produced by phytoplankton in the Earth's surface oceans annually[9]. However, surface sediment from saltmarsh ponds, an estuary and the deep ocean (with high pressures and no light) contained DMSP levels (5-128 nmol DMSP g^{-1}) that were up to ~three orders of magnitude higher than the overlying seawater (0.01-0.70 nmol DMSP ml^{-1}) (Fig. 1a-b, Supplementary Tables 1a and 2), a phenomenon also observed in **10,11**. DMSP concentration decreased with depth, being much lower in anoxic sediment, but even in deeper sediments the concentration was approximately an order of magnitude higher than in the overlying seawater (Supplementary Table 1a). This study focused on DMSP synthesis in coastal surface sediments, where DMSP concentrations were highest. The

DMSP-producing cordgrass *Spartina* is proposed to be the major DMSP and DMS source in many saltmarshes[12,13]. Indeed, high DMSP levels were found in *Spartina anglica* roots and leaves around the sampled ponds, and the highest sediment DMSP levels detected were adjacent to this cordgrass (Supplementary Fig. 1a-b). However, *S. anglica* rhizosphere and phyllosphere samples contained bacteria with the genetic potential to synthesise DMSP (Supplementary Table 3), and we cannot dismiss the possible contribution of bacteria to DMSP levels in *S. anglica* and/or the surrounding environment. Furthermore, surface sediment DMSP concentrations stabilise ~20 cm away from the *Spartina* (Supplementary Fig. 1b). Yarmouth estuary, which also had high DMSP levels (Supplementary Table 1a), lacked *Spartina* and was populated with *Aster tripolium*, a halophyte not known to accumulate DMSP, but which contained DMSP, at lower levels than *S. anglica* (Supplementary Fig. 1a). As with DMSP standing-stock concentrations, DMSP and DMS synthesis rates were much higher in surface sediment than the overlying water samples (Table 1, Supplementary Fig. 2). These data suggest that a sizeable amount of DMSP in the sediment may result from microbial biosynthesis, rather than solely from sinking particles or DMSP-producing plants. We propose that surface coastal and marine sediments in general, which cover >70% of the Earth's surface[14], are highly active environments for microbial DMSP biosynthesis and catabolism, generating the climate-active gas DMS.

Microbial community analysis was performed on Stiffkey saltmarsh surface sediment to identify potential DMSP-producers. This was dominated by bacteria (~91% of 16S rRNA gene sequences {Supplementary Fig. 4}), of which ~2.3 ±0.6% belonged to genera that include *dsyB*-containing species, a reporter gene for bacterial DMSP synthesis[8] (Supplementary Figs. 5 and 6, Supplementary Tables 4 and 5). Furthermore, metagenomic analysis predicted that ~1% of bacteria contain *dsyB*, spanning functional methylthiohydroxybutyrate (MTHB) methyltransferases[8] (Supplementary Fig. 7). This

abundance was higher than most DMSP lyases (enzymes that cleave DMSP, releasing DMS) apart from the genes encoding DddD, DddL and DddP (present in 1.1, 4.8 and 6.6% of bacteria, respectively) (Supplementary Table 7), which are likely important DMS-producing enzymes in these sediments. Eukaryotic plastid 16S rRNA genes, predominately from diatoms, represented ~9% of the community sequences (Supplementary Fig. 4). *Asterionellopsis*, a member of the Fragilariophyceae family with low or undetectable intracellular DMSP levels[15], was the most abundant diatom (~6% of 16S rRNA gene community data {Supplementary Fig. 5}). A 3 μm diameter chain-forming *Asterionellopsis glacialis* (strain PR1) isolated from Stiffkey sediment, with 99% 16S rRNA gene identity to the dominant *Asterionellopsis* in the amplicon data, produced low intracellular DMSP levels (0.21 mM) (Supplementary Fig. 8). No DMSP synthesis genes have been identified in the Fragilariophyceae family of diatoms, but in Stiffkey metagenomes the eukaryotic DMSP synthesis gene *DSYB*[16] was ~13-fold less abundant than *dsyB* (Supplementary Table 7). The plastid 16S rRNA gene sequences of other DMSP-producing eukaryotes were detected at very low levels, including *Phaeodactylum* (0.4%) and *Thalassiosira* (0.3%). Given the abundance of DMSP-producing diatoms and bacteria in Stiffkey sediments, both are likely important DMSP producers in such photic marine environments.

Incubation experiments were conducted to enrich for and isolate DMSP-producing bacteria from Stiffkey sediment. DMSP production in 'enriched' sediment slurries was enhanced by incubation in enrichment media with increased salinity, MTHB addition, and reduced nitrogen levels (conditions enhancing DMSP synthesis in *Labrenzia*[14]). Over 14 days DMSP levels were consistently highest (day 4 onwards) in microbial particulates from enriched samples (Supplementary Fig. 9). The proportion of DMSP-producing bacterial isolates (Supplementary Table 8) in the enriched sediment increased to 71%, from 25% in natural

sediment. This supports these incubation experiments as an effective enrichment methodology for DMSP-producing bacteria.

Enriched and control microbial community profiles greatly differed from those in natural sediment, likely due to the addition of media, mixed carbon source and/or other differences from the natural conditions (temperature, dissolved oxygen, etc) during the incubation (Supplementary Figs. 4, 5, 7 and 10). Importantly, the enriched microbial community had several clear genus-level differences to the natural and control samples. The abundance of known DMSP-producers *Oceanicola* (2.1 ±0.01%) and *Ruegeria* (4.5 ±0.1%) significantly increased in the enriched samples (Supplementary Figs. 5 and 10), alongside genera of DMSP-producing alpha- and gammaproteobacterial isolates from this study, e.g., *Marinobacter* (3.2 ±0.4%), *Novosphingobium* (4.7 ±0.9%) and *Alteromonas* (20.7 ±2.4%). Bacteria of the latter three genera, comprising ~0.6% of the natural sediment community and lacking *dsyB* in their available genomes, likely contributed to the enhanced DMSP levels seen in enriched samples (Supplementary Fig. 9). The abundance of *dsyB*-containing bacterial genera (11.7 and 10.5%, Supplementary Table 5), the *dsyB* gene (determined by qPCR and metagenomics {Fig. 1c, Supplementary Table 7}), and *dsyB* transcripts (Fig. 1c) showed no significant differences between control and enriched samples. However, the DsyB diversity varied somewhat between the two (Supplementary Fig. 6). It is possible that bacteria with the more abundant DsyB variants in the enriched samples may contribute to the higher observed DMSP levels, e.g. by producing higher intracellular DMSP concentrations. Alternatively, there may be additional unknown DMSP synthesis genes/pathways contributing to the enhanced DMSP levels seen.

Novosphingobium sp. BW1 was used to investigate *dsyB*-independent DMSP production pathways. Of the known DMSP synthesis pathway intermediates (Fig. 2a)[17], BW1 DMSP

production was significantly enhanced by adding methionine (Met), the universal DMSP precursor, and *S*-methyl-methionine (SMM), a common plant metabolite[18,19] and intermediate of the methylation pathway in DMSP-producing plants, e.g., *Spartina*[20] (Fig. 2b). Met (0.90 ±0.01 mM) and another intermediate in this pathway, DMSP-amine (0.13 ±0.02 mM), were detected in BW1 cell extracts by HPLC (Supplementary Fig. 11) and SMM was detected by LC-MS, further supporting the methylation pathway as the likely BW1 DMSP synthesis pathway. DMSP-amine addition did not enhance DMSP production, possibly due to the ability of BW1 to import DMSP-amine, or because DMSP-amine may not induce the expression of DMSP synthesis genes. BW1 cell extracts had *S*-adenosyl-Met (SAM)-dependent Met methyltransferase (MMT) activity, converting Met to SMM (3.6 μmol min^{-1} μg $protein^{-1}$). Although some bacteria catabolise SMM[19,21] for use as a methyl donor, none have previously been shown to possess MMT activity. Addition of 4-methylthio-2-oxobutyrate (MTOB) also enhanced BW1 DMSP production (2.5-fold), but to a lesser extent than Met or SMM (7- and 13-fold, respectively), perhaps indicating that BW1 has an active Met salvage pathway generating Met from MTOB[22].

By screening a BW1 genomic library, a gene conferring MMT activity (EC2.1.1.12) termed *mmtN* was identified (Supplementary Table 8, Supplementary Fig. 12). Purified MmtN had SAM-dependent MMT activity, but did not methylate related compounds, including MMPA, glycine and MTHB (Supplementary Figs. 13a and 14). MmtN homologues (≥ 54% aa identity), exist in many marine alphaproteobacteria, one gammaproteobacterium and some actinobacteria, representatives of which produced DMSP, with *mmtN*-like genes that were cloned and functionally ratified (Fig. 3, Supplementary Fig. 12, Supplementary Table 8). A recent biochemical study characterised MmtN from *S. mobaraensis* and *Rhodovulum* sp. P5 as having MMT activity[23], with K_M values comparable to those reported here for *Novosphingobium* MmtN (Supplementary Fig. 14).

145 Thus, *mmtN*, like *dsyB*[8] and *DSYB*[16], is another robust reporter gene for an organism's
146 potential to synthesise DMSP. *mmtN*-containing bacteria were less abundant than those with
147 *dsyB* in tested seawater and sediment samples (Fig. 1c, Supplementary Tables 5 and 7).
148 However, the abundance of *mmtN*-containing bacteria was higher in the enriched versus
149 control incubation samples, suggesting that MmtN-dependent DMSP production may be a
150 significant contributor to the increased DMSP levels seen under the enrichment conditions
151 (Fig. 1c, Supplementary Tables 5 and 7).

152 The *mmtN* gene is required for DMSP synthesis in *T. profundimaris,* since an *mmtN*$^-$ mutant
153 did not produce DMSP, and was restored by complementation with cloned *mmtN* (Fig. 2c,
154 Supplementary Table 8, Supplementary Fig. 13b). Further work is required to elucidate the
155 complete MmtN-dependent DMSP synthesis pathway, which likely involves a suite of genes
156 (two distinct types) encoding a putative aminotransferase, dehydrogenase and decarboxylase
157 adjacent to *mmtN* in many marine bacterial genomes (Supplementary Fig. 12). Liao and
158 Seebeck[23] found that *S. mobaraensis* candidate gene products from one such suite of genes
159 (SMM decarboxylase, DMSP-amine aminotransferase and DMSP-aldehyde dehydrogenase
160 {Figure 2a}) had the expected enzyme activities. We also show that mutation of the putative
161 DMSP-amine aminotransferase (TH2_03140), part of the second suite of genes
162 (Supplementary Fig. 12) in *T. profundimaris,* caused a 73% reduction in DMSP compared to
163 wild type *T. profundimaris.* This suggests that at least one of these linked genes encodes a
164 downstream enzyme in the DMSP biosynthesis pathway in *T. profundimaris*. The *mmtN*$^-$
165 mutant displayed no significant growth reduction or competitive disadvantage compared to
166 the wild type strain in response to increased salinity and/or reduced nitrogen conditions,
167 which were known to enhance DMSP production in this bacterium (Supplementary Fig. 15).
168 Similar results were found with a *Labrenzia dsyB*$^-$ mutant[8], which, like *T. profundimaris,* also
169 produces the nitrogenous osmolyte glycine betaine (GBT). Indeed, the *T. profundimaris*

mmtN⁻ mutant displayed enhanced GBT production levels compared to the wild type, suggesting that GBT, and/or other osmolytes produced by these bacteria, compensate for the loss of DMSP (Fig. 2c, Supplementary Fig. 13b).

MmtN proteins form a distinct group (Fig. 3), but have ≤30% identity to the N-terminal methyltransferase domain of distantly related and larger (33 versus 115 kDa) plant Met *S*-methyltransferase MMT enzymes (PLN02672) (Fig. 3). These contain an extra C-terminal aminotransferase domain (pfam00155) thought to have a regulatory role[24]. The amino acid and domain differences between the bacterial MmtN and plant MMT enzymes are likely responsible for the ~10-fold higher K_M values observed for the former[24]. Genes encoding full-length plant-like MMT enzymes exist in some bacterial genomes, mainly deltaproteobacteria (Fig. 3), and four such bacteria were tested for DMSP production. Only *Pseudobacteriovorax antillogorgiicola* DSM103413 produced DMSP, at low levels (Supplementary Table 8). Thus, unlike *mmtN*, the presence of the full-length plant-like MMT in an organism is not a good indicator of DMSP production. Within the group containing functional MmtN proteins, we did not find monophyly among the major bacterial groups, suggesting that *mmtN* may have transferred between bacteria by horizontal gene transfer. The high level of sequence divergence between bacterial *mmtN* and full-length MMT genes suggests that this pathway is ancient, arising independently in bacteria and plants, or possibly through ancient horizontal gene transfer.

DMSP-producing bacteria (containing DsyB and/or MmtN) are predicted by qPCR to constitute 0.1-3.6% of bacteria in the tested marine sediment samples, from saltmarsh ponds, an estuary and the deep ocean (Supplementary Table 9). Indeed, the percentage of DMSP-producers predicted by metagenomic analysis is ~1.1% (Supplementary Table 7), which, when applied to the estimated 1.99×10^{10} bacterial cells g sediment^{-1} in Stiffkey surface

194 sediment (Supplementary Table 10) suggests an abundance of ~10^8 DMSP-producing
195 bacteria g sediment^{-1}, with intracellular DMSP levels ranging from 0.66–73 mM (Fig. 1c,
196 Supplementary Tables 7 and 9). DMSP-producing bacteria were much less abundant in the
197 ocean microbial reference gene catalogue metagenomic database (OM-GRC)[25]
198 (predominantly surface seawater samples) and in tested coastal seawater samples compared
199 to the surface sediment, but they still represent 0.3-0.6% of a reported 10^6 bacteria ml^{-1} in
200 seawater[26] (Fig. 1c, Supplementary Tables 7 and 9). These predictions are likely
201 underestimations, since some isolated bacteria lacking *dsyB* and *mmtN* were shown to
202 produce DMSP (e.g., *Marinobacter,* representing ~0.5% of the natural sediment community),
203 probably via unidentified DMSP synthesis genes/pathways. The *dsyB* gene was transcribed in
204 all tested samples, but was > three orders of magnitude higher, per unit mass, in surface
205 sediment than in pond water and surface seawater (Fig. 1c). Furthermore, *dsyB*[16] and *mmtN*
206 transcripts are omnipresent or mostly present, respectively, at varied levels in *Tara* Oceans
207 bacterioplankton metatranscriptome databases (Supplementary Tables 11 and 12). In
208 seawater incubation experiments *Novosphingobium* sp. BW1 (*mmtN*$^+$), *Pelagibaca*
209 *bermudensis* (*dsyB*$^+$) and *Labrenzia* LZB033 (*dsyB*$^+$) produced DMSP and contributed to the
210 dissolved pool, demonstrating activity under closer to natural conditions (Supplementary Fig.
211 16, Supplementary Table 13). These data are consistent with a large global biomass of
212 DMSP-producing bacteria actively synthesising DMSP in marine sediment and seawater
213 environments. This work shows that bacteria likely contribute to DMSP levels in seawater
214 environments, but further work is required to evaluate their significance. Additionally, the
215 contribution of bacteria, and in some cases that of benthic algae, to total DMSP levels is
216 likely to be far higher in marine surface sediments, which per unit mass are more productive
217 than the overlying seawater. Moreover, while the DMSP content in the anoxic saltmarsh
218 sediment is far lower than the oxic surface layer (Supplementary Table 1a), it is still ~5- to

219 10-fold higher than that of the overlying seawater, and is an environment in which bacterial
220 DMSP production is unstudied. This study challenges the notion that DMSP production is
221 mainly an oxic and photic process and suggests that global models for DMSP and DMS
222 production should consider marine surface sediments and bacteria as important contributors.

Methods

General Scientific Practices

Chemical syntheses

DMSP was synthesised from DMS (Sigma-Aldrich) and acrylic acid (Sigma-Aldrich) as described in Todd *et al.*[27]. DMSHB, DMSP-amine and SMM were synthesised as in Curson *et al.*[8]. Met, MTOB, MTHB and MTPA are commercially available and were obtained from Sigma-Aldrich.

Quantification of DMS/DMSP/SMM by GC

All gas chromatography (GC) assays involved measurement of headspace DMS, either directly produced or via alkaline lysis of DMSP or SMM, using a flame photometric detector (Agilent 7890A GC fitted with a 7693 autosampler) and a HP-INNOWax 30 m × 0.320 mm capillary column (Agilent Technologies J&W Scientific). Unless otherwise stated, all DMSP/SMM GC measurements were performed using 2 ml glass vials containing 0.3 ml liquid samples and sealed with PTFE/rubber crimp caps. To quantify DMSP, 0.2 ml of overnight culture was added to a 2 ml vial, 0.1 ml 10 M NaOH was added, vials were crimped immediately, incubated at 22°C overnight in the dark and monitored by GC. To quantify SMM production, 0.2 ml of culture and 0.1 ml of 10 M NaOH were sealed in glass vials and heated at 80°C for 10 min before incubating overnight and sampling. An eight-point calibration curve was produced by alkaline lysis of DMSP and SMM standards in water. The detection limit (per 300 µl sample in 2 ml GC vial) was 0.015 nmol for DMSP and 1.5 nmol for SMM.

Detection of DMSP, GBT and SMM by LC-MS

LC-MS was used to confirm that bacteria were producing DMSP and at similar levels to those determined by GC, ruling out the possibility that DMS detected by GC was due to some other compound and not DMSP. The method used for the detection of DMSP and GBT was as described in Curson *et al*.[8]. SMM detection followed the same method. All samples (15 µl) were analysed immediately after being extracted. The targeted mass transition corresponded to $[M+H]^+$ of DMSP (*m/z* 135), GBT (*m/z* 118) and SMM (*m/z* 165) in positive mode. To confirm the presence of the compounds in the biological samples, standards were also run (10-50 µM).

Quantification of Met, DMSP and SMM by HPLC

HPLC methods were developed to determine Met, SMM and DMSP-amine as fluorescent adducts after pre-column derivatisation with ortho-phthaldialdehye (OPDA)[28], but employing mercaptoethanol, instead of mercaptopropionic acid, as the thiol reagent. Samples (50 µl) were mixed with 50 µl derivatisation reagent (5 mg OPDA in 5 ml methanol, buffered with 35 ml 1 M potassium borate buffer, pH 10.4, mixed with 84 µl mercaptoethanol), and reacted for 3 min before injecting a 10 µl sample onto a 4.6 x 250 mm Synergi Hydro-RP (Phenomenex) column, eluted according to Caddick *et al* [28]. Fluorescent adducts were detected with a Jasco FP-920 fluorescence detector set at Ex 332 nm, Em 445 nm, with bandpass 18 nm and gain 10. Met, SMM and DMSP-amine standards yielded correlation coefficients of >0.999, >0.999 and >0.995, respectively, for 5-point calibration in the range 1-20 µM. A 5-point calibration for Met in the range 0.2-2 µM in seawater media yielded a correlation coefficient >0.984. The limit of detection of Met, at 3x noise, of the chromatogram, was estimated to be c. 0.02 µM in samples. Seawater was filtered with a 0.45 µm syringe filter. For detecting dissolved Met in the sediment, 0.3 g sediment was diluted

with 3 ml ESAW artificial seawater medium then centrifuged at 500 *g* for 20 minutes. The supernatant was removed and filtered with a 0.45 µm syringe filter before analysing for Met.

Quantification of DMSP by purge trap

Total DMSP samples of seawater and sediment were fixed with 50% (v/v) H_2SO_4 and stored at room temperature for 2 days. For seawater samples, 250 µl 50% H_2SO_4 was directly added to 25 ml of seawater and then sealed. For sediment samples, 0.5 g of sediment was first mixed with 25 ml distilled water, then added to 250 µl 50% H_2SO_4 and sealed. For analysis, 1 ml of 10 M NaOH was injected into 5 ml of the preserved seawater sample and then sealed and incubated in the dark at 22°C for 16 h. To measure DMSP in sediment, the samples were centrifuged at 5,000 *g* and 5 ml of the supernatant of the preserved mix was used. The liberated DMS was measured using the purge and trap method[29]. Briefly, sulfur gases were sparged from the sample with nitrogen and trapped in a loop of tubing immersed in liquid nitrogen. The trapped gases were desorbed with hot water (above 90°C) and analysed by GC.

The DMSP content of seawater was determined by taking 25 ml seawater mixed with H_2SO_4 (to 0.5%). This mix was incubated at room temperature for 2 days and 5 ml was then mixed with 1 ml 10 M NaOH and incubated at 22°C for 16 h in the dark, before using a modified purge and trap method as described in Zhang *et al*.[30] to collect the DMS released by the sample. The samples were purged for 20 mins and then compounds were detected by GC.

Site Characterisation

Environmental parameters of Stiffkey saltmarsh

The oxygen saturation was measured at the water surface, half depth (80 mm) and above the water/sediment interface (160 mm) using a Jenway 970 and a 2-point calibration with filtered

seawater in equilibrium with air (100% oxygen saturation) and a 2 M sodium sulfite solution (0% oxygen saturation). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) measurements were made using triplicate measurements by a Skalar Formacs CA15 analyser, employing a six-point calibration. TDN represents the sum of all dissolved nitrogen-containing species (excluding dinitrogen {N_2}) and includes organic nitrogen species as well as nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and nitrous oxide (N_2O).

Nutrient analysis was performed using a Seal AA3 AutoAnalzyer at CEFAS, Lowestoft. Phosphate was measured as described in Murphy and Riley[31], ammonium as in Jones[32] and nitrate and nitrite as in Armstrong *et al*.[33].

Sampling sediment

The majority of the enrichment and isolation work described in this study was performed on surface sediment samples from Stiffkey saltmarsh, UK (52.9643, 0.9255) (Supplementary Table 1a-b). Triplicate marine sediment samples were collected using sterile acrylic corers at least 40 cm from the banks of the ponds. DMSP content was measured in samples taken from the overlying water (200 µl), which was ~15-20 cm deep, from the surface sediment layer (top 1 cm) and from three anoxic depths (5 cm, 10 cm and 15 cm). Cores were transported immediately to the laboratory and processed on arrival. Surface sediment and water (~20-30 cm deep) from Cley saltmarsh, UK (52.9586, 1.0473) and Yarmouth Estuary, UK (52.6133, 1.7162) were also sampled for comparison of DMSP production rates as well as expression of key genes involved in DMSP synthesis. Finally, the R/V Dong Fang Hong 2 cruise (September 2016) sampled surface seawater and deep-sea surface sediment (4,500 m depth) from the Mariana Trench (10.4091, 142.3569) using a box corer.

DMSP/DMS rate experiments with ^{3}H-methionine

313 Experiments to establish rates of DMS/DMSP production in surface sediment from Stiffkey,
314 Yarmouth and Cley were undertaken as follows. For seawater samples, 10 ml seawater was
315 added to a 30 ml universal bottle. For sediment samples, 1 g sediment and 10 ml autoclaved
316 seawater was added to a 30 ml universal bottle. Autoclaved sediment and seawater were used
317 as negative controls. L-[methyl-^{3}H]-methionine (85 Ci mmol^{-1}; Perkin Elmer) was added to a
318 final concentration of 6 nM (185 kBq) and samples were incubated at 22°C for the times
319 specified. For DMSP measurements, at each timepoint (T=30, 60, 90, 180, and 240 min) 1 ml
320 of seawater or sediment/seawater slurry were removed to a new 30 ml universal bottle
321 containing 13 µl of 20% (v/v) H_2SO_4 (to prevent further bacterial activity, stabilise the DMSP
322 and convert ^{3}H volatiles to non-volatiles) and mixed. A 1.5 ml centrifuge tube containing a
323 DMS trap was placed in the top of the universal bottle. The DMS trap consisted of half a 25
324 mm GF/F glass microfibre filter folded and soaked in 100 µl 3% (v/v) hydrogen peroxide to
325 collect headspace DMS, as in Slezak *et al.*[34]. To release the ^{3}H-DMS from any ^{3}H-DMSP
326 present, 1 ml of 10 M NaOH was added to the seawater/slurry in the universal bottle
327 containing the trap. Universal bottles were sealed, shaken gently and incubated at 22°C for 24
328 h to trap ^{3}H-DMS. Filters were removed to a 20 ml polyethylene vial containing 4 ml
329 scintillant (Ecoscint A) and the vials mixed. Samples were left in the dark for 1 h before
330 scintillation counting on a Hidex 300 SL scintillation counter. DMS measurements were done
331 in the same way as described for DMSP except that the DMS trap was placed directly into the
332 universal bottle containing the seawater or sediment slurry without added NaOH. This
333 allowed DMS produced and released into the headspace, through microbial cleavage of any
334 ^{3}H-DMSP, to be captured in the trap. Filters were removed after 24 h and ^{3}H measured as for
335 the DMSP samples. Counts per minute values recorded were used to calculate the rate of
336 DMSP/DMS production expressed as fmol g^{-1} min^{-1} or fmol ml^{-1} min^{-1} for sediment or

337 seawater samples respectively and DMS production in sediment over a 24 h period expressed
338 as nmol g^{-1}.

339 Rates of DMSP or DMS production were calculated based on the amount of labelled product
340 produced (as ^{3}H-DMS). For DMSP production rates in sediment, experiments were done with
341 the labelled ^{3}H-Met substrate in tracer amounts (< 0.6%) relative to the dissolved ambient
342 Met concentration, estimated here to be 3.94 ±0.89 μM for Stiffkey, 2.71 ±0.20 μM for Cley,
343 and 1.04 ±0.88 μM for Yarmouth[35]. The rate derived from the labelled product was then
344 multiplied according to the factor of dissolved ambient methionine concentration relative to
345 the added labelled ^{3}H-Met concentration (6 nM). For DMSP production rates measured in
346 Stiffkey seawater, dissolved ambient Met was 0.34 ±0.06 μM, and calculations were made as
347 for DMSP in sediment above to correct by the factor of dissolved ambient methionine relative
348 to labelled ^{3}H-Met added. This value for dissolved ambient Met in seawater was used for all
349 sites. The values were converted to pmol DMSP m^{-2} h^{-1} and pmol DMSP cm^{-3} h^{-1} for
350 sediment and seawater respectively by normalising wet to dry sediment using a factor of 0.5 g
351 cm^{-3}, determined in weight/drying measurements[36] on sediments comparable to those of
352 Stiffkey. Finally, it was assumed that this type of active, oxic sediment makes up the top 1 cm
353 of sediment, converting rates cm^{-3} to rates m^{-2}.

354 For measurements of DMS produced from dissolved ambient Met over 24 hours in sediment,
355 these values were calculated from the labelled ^{3}H-DMS produced. As with experiments for
356 DMSP production rates in sediment described above, labelled ^{3}H-Met was used as substrate
357 in tracer amounts (< 0.6%). The amount of labelled ^{3}H-DMS produced was corrected by the
358 factor of the dissolved ambient methionine concentration in sediment at each location (see
359 above) relative to the added labelled ^{3}H-DMSP concentration (6 nM).

These DMSP and DMS production rate estimations are performed under lab conditions that do not consider the ambient Met already within cells, thus, we advise caution in their extrapolation beyond this level.

DMSP cleavage rate experiments

Experiments to approximate the rate of DMSP catabolism generating DMS in surface sediment and overlying pond or seawater from Stiffkey, Yarmouth and Cley were performed as follows. For pond or seawater samples, triplicate 10 ml samples were added to a 140 ml serum vial. For sediment samples, 1 g sediment and 10 ml autoclaved seawater was added to a 140 ml serum vial in triplicate. DMSP was added to a final concentration of 0.1 mM alongside controls with no DMSP, and vials were crimp-sealed immediately. DMS headspace concentrations were measured at T=0, 30, 60, 90 and 120 min by GC (see above) using manual injections. These measurements were used to calculate the rate of DMS production, expressed as nmol g^{-1} min^{-1} or nmol ml^{-1} min^{-1} for sediment or seawater samples respectively.

DMSP analysis on *Spartina anglica,* the surrounding surface sediment and *Aster tripolium*

Plant and sediment samples were taken during low tide from ponds in Stiffkey and Cley saltmarsh and from Yarmouth estuary. *A. tripolium* and *S. anglica* plants were carefully uprooted and placed in sterile plastic bags. Surface sediment from Stiffkey was sampled as above, following a 100 cm transect moving away from *Spartina,* sampling every 10 cm. Plant material was washed to remove sediment and separated into different tissues (roots & shoots/leaves for *S. anglica* and stems and leaves for *A. tripolium*) using ethanol sterilised scissors and tweezers. The phyllosphere and rhizosphere of *S. anglica* were sampled by washing 10 g leaves and 5 g roots in 10 ml sterile water with vortexing for 5 min, and

repeating five times. The five washates were centrifuged for 10 min at 15,000 *g* and DNA was extracted from the pellets. This DNA was used as a template for qPCR analysis to test for the presence and abundance of *dsyB* and *mmtN*, with the values from each of the five washates being pooled to give total phyllosphere and rhizosphere gene abundance, normalised to the weight of plant tissue washed (Supplementary Table 3).

Between 1-5 g (fresh weight) of tissue was ground to fine powder particles with liquid nitrogen using a pre-cooled sterile ceramic mortar and pestle. To measure DMSP content, approximately 0.1 g (fresh weight) of the ground material was added to 2 ml glass GC vials and 300 µl 10 M NaOH was immediately added and vials were sealed with 11 mm crimp caps with rubber/PTFE septa and mixed. For the transect samples, 10-20 g of sediment was mixed thoroughly to ensure a homogenous sample. Replicates of ~0.1 g (wet weight) of this mix were weighed into GC vials and mixed with 300 µl 10 M NaOH before crimp-sealing, as above. Samples were left overnight in the dark at 22°C before GC analysis (see 'Quantification of DMS/DMSP/SMM by gas chromatography').

Isolation of *Asterionellopsis glacialis*

In order to isolate epipelic diatoms present on the surface of saltmarsh pond sediment, samples were taken by scraping the top 0.5-1 cm surface layer of the sediment. These were then subsampled and inoculated into 250 ml flasks containing F/2 medium (made with 0.2 µm-filtered sterile Stiffkey pond water, 32 practical salinity units {PSU}); Guillard and Ryther [37]). Several monoclonal isolates of pennate diatoms, including *Asterionellopsis*, were established using the single-colony isolation technique described in Andersen *et al*.[38]. Isolates were allowed to grow for 2-3 weeks at a constant temperature of 22°C under a 12:12h light:dark photoperiod with a constant photon flux of 120 µE $m^{-2}s^{-1}$ (QSL-100 Quantum

406 Scalar Irradiance Meter, Biospherical Instruments, San Diego, USA) provided by Philips
407 MASTER TL-D 58W/840 white tubes. Isolates from enriched cultures were then further
408 purified and unnecessary contaminating picoplankton were removed by dilution. Once
409 purified, strains were transferred to 42-well plates and allowed to grow for approximately 2-3
410 weeks. Cultures were treated with multiple rounds of antibiotic treatment (400 μg ml^{-1}
411 streptomycin, 50 μg ml^{-1} chloramphenicol, 20 μg ml^{-1} gentamicin and 100 μg ml^{-1} ampicillin)
412 to remove as many bacteria as possible. Clonal cultures were then transferred and up-scaled
413 to culture flasks (Nunc™ EasYFlask with Filter Caps, 75 cm^2 cell culture area, Thermo
414 Fisher Scientific) containing 20 – 40 ml F/2 medium (0.2 μm-filtered sterile 50:50 pond
415 water and ESAW artificial seawater, 35 PSU). The isolate used in this study was a strain
416 termed *Asterionellopsis glacialis* strain PR1. For culturing *A. glacialis* PR1 for DMSP
417 quantification, 30 ml of stock culture (3 x 10^5 cells ml^{-1}) was inoculated to 200 ml F/2
418 medium (made with ESAW artificial seawater medium, 35 PSU) in triplicate. Growth was
419 monitored every day by cell counting with a Zeiss Primovert inverted optical microscope and
420 a Sedgewick-Rafter counting cell. Cells were harvested after 24 days (in stationary phase,
421 Supplementary Fig. 8) and assayed for DMSP as in Curson *et al.*[16]. The cell volume of *A.*
422 *glacialis* PR1 used for intracellular DMSP calculations was 654 μm^3 and this was based on
423 calculations as in Naz *et al.*[39].

424 **DNA extraction and PCR amplification of rRNA genes from *A. glacialis* PR1**

425 PR1 cells were harvested by centrifuging 100 ml of culture containing 3.34 x10^5 cells ml^{-1} for
426 10 minutes at 5,000 *g* and genomic DNA was extracted as described in Yin *et al.*[40], with the
427 following modifications. Cell disruption was achieved through bead beating at 6 m s^{-1} for 60
428 s with a Bead blaster 24 bead beater (Benchmark, Edison, NJ, USA), using silica beads
429 (Lysing Matrix E, MP Biomedicals, Cambridge, UK) in 60 μl of 10% (w/v) sodium dodecyl

sulfate (SDS). After cell disruption, 6 µl of proteinase K (10 mg ml^{-1}) was added to the sample and incubated for 20 mins at 65°C, then centrifuged at 15,000 *g* for 10 mins. Nucleic acid extracts were precipitated in an equal volume of cold isopropanol and washed with 800 µl cold 75% ethanol, and the pellets dissolved in 100 µl nuclease-free water and stored at -80°C. The 16S and 18S rRNA genes were PCR amplified using primers 8F/1492R and primers Euk_A/Euk_B primers, respectively. PCR was carried out, the products were cloned into pGEM-T easy (Promega), sequenced and analysed (see 'General *in vivo* and *in vitro* genetic manipulations').

Culture-independent work

Enrichment to enhance DMSP production in Stiffkey sediment

Microcosm experiments were set up to increase DMSP production and abundance of DMSP-producing organisms from Stiffkey saltmarsh sediment. Microcosms consisted of 2 g of surface sediment slurries in 30 ml MBM media of varying compositions, including a control with MBM (35 PSU, 10 mM NH_4Cl), high salinity (50 PSU), low nitrogen (0.5 mM NH_4Cl), additional MTHB (0.1 mM) or a combination of all three conditions (50 PSU, 0.5 mM NH_4Cl, 0.1 mM MTHB). Samples were incubated at 28ºC for 7 days before quantifying DMSP content (Supplementary Fig. 9). This experiment was scaled up for molecular microbial ecology work using 3 g sediment and 45 ml MBM with either the combined conditions (enriched media; 50 PSU, 0.5 mM NH_4Cl, 0.1 mM MTHB) or control MBM (35 PSU, 10 mM NH_4Cl). Sediment slurries were incubated at 28ºC for 14 days. Samples were taken at regular time points, centrifuged and the DMSP content determined in the particulate and cell-free medium (Supplementary Fig. 9). All experiments were done in triplicate.

DNA/RNA extraction and purification

453 DNA and RNA were extracted from all marine sediment samples (Time 0) and from the 14
454 day incubation sediment (enriched and control samples, see above) following the protocol
455 described by Carrión *et al.*[41]. Samples were stored at -80°C and RNA was purified separately
456 (see below).

457 **Degenerate primer design**

458 To design degenerate primers targeting the *dsyB* gene, 24 DsyB sequences available from
459 Genbank were aligned using the ARB[42] project program to identify conserved amino acid
460 positions. Two non-DsyB sequences with a cut-off value below that used in Curson *et al*.[8,16]
461 were also included in the alignment to guide specific amplification of *dsyB* by the degenerate
462 primers **(**Supplementary Fig. 17a**)**. Various sets of primers with a degeneracy ≤5 bp spanning
463 different regions of the *dsyB* gene were manually designed. Several different combinations
464 were tested against genomic DNA from positive and negative control strains (Supplementary
465 Table 15). The primer pair dsyB_deg1F and dsyB_deg2R (Supplementary Table 17) yielded
466 a 246 bp fragment from genomic DNA from all positive controls strains tested, with no non-
467 specific bands, and no amplification bands at that size were obtained from any of the negative
468 control strains **(**Supplementary Fig. 17B**)**. Optimisation of PCR conditions with these primers
469 included annealing temperatures ranging from 60 - 65°C, extension times from 15-60 s and
470 30-40 of cycles. The most specific amplification was obtained with an initial denaturation
471 step of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, an annealing step of 61°C for
472 15 s and an elongation step of 72°C for 15 s, with a final extension of 72°C for 5 min.

473 Degenerate primers for *mmtN* were designed following the same principles as above with the
474 j-CODEHOP PCR primer design programme[43] using 20 MmtN sequences from Genbank and
475 one MMT sequence (primers were designed to amplify bacterial *mmtN*) (Supplementary Fig.
476 17C). The primers mmtN_degF and mmtN_degR were selected for further analysis

(Supplementary Table 17) since they yielded a product of the expected size (301 bp) from four positive control strains, showed the least number of unspecific bands and did not amplify the negative control (Supplementary Fig. 17D). PCR conditions for these primers were optimised using annealing temperatures between 50-60°C, extension times ranging from 20-45 s and 30-35 cycles. The final PCR program consisted of an initial denaturation step of 95°C for 3 min, 35 cycles of 95°C for 20 s, annealing at 54°C for 30 s and elongation at 72°C for 30 s, ending in a final extension of 72°C for 7 min.

Quantitative PCR and reverse transcription qPCR (RT-qPCR)

To study the abundance of *dsyB* and *mmtN* transcripts, RNA from environmental samples was purified using the RNase-free kit (Qiagen) and the RNeasy mini kit (Qiagen) according to the manufacturer's instructions. Absence of DNA in RNA samples was confirmed by PCR using primers 27F/1492R[44] (Supplementary Table 17). Purified RNA samples were quantified with a Qubit RNA HS assay kit (Thermo Fisher Scientific). Reverse transcription of RNA was performed with ~100 µg purified RNA. Between 1-9 µl RNA were mixed with 1 µl 10 µM specific reverse primer (Supplementary Table 17) as in Farhan Ul Haque *et al* [45]. The mixture was incubated for 5 min at 70°C and cooled briefly on ice. Then, 1 µl dNTPs (10 mM), 4 µl M-MLV 5 x reaction buffer (Promega), 0.4 µl RNase Inhibitor (40 U/µl, Roche), 0.8 µl M-MLV reverse transcriptase (200 U/µl, Promega) and 3.8 µl nuclease-free water were added to the mixture. Finally, samples were incubated at 42°C for 1 h and resultant cDNA was stored at - 20°C until use.

qPCR and RT-qPCR assays were performed using a C1000 Thermal cycler equipped with a CFX96 Real-time PCR detection system (BioRad). qPCR reactions (20 µl) contained 2 µl of cDNA/DNA (2-10 ng for 16S rRNA gene and 10-50 ng for *dsyB/mmtN*), 0.8 µl of each primer (10 µM) and 10 µl of SensiFAST™ SYBR® Hi-ROX Kit (Bioline). The primers used

in qPCR/RT-qPCR are described in Supplementary Table 17. The qPCR and RT-qPCR reactions consisted of an initial denaturation step of 95°C for 3 min, followed by 40 cycles of 95°C for 20 s, 55°C (16S rRNA gene)/60°C (*dsyB*)/54°C (*mmtN*) for 20 s and 72°C for 30s. Specificity of qPCR and RT-qPCR reactions was determined from melting curves from 60-95°C, followed by gel electrophoresis and clone library construction from DNA and/or cDNA isolated from environmental samples. Ratified sequences were between 77-100% identity at the derived amino acid level to ratified DsyB proteins[8,16], and 63-73% to ratified MmtN proteins (Supplementary Table 14), respectively.

Quantification of *dsyB* and *mmtN* genes/transcripts was performed using a ten-fold dilution series of DNA/cDNA standards. Standards were prepared by cloning the *dsyB/mmtN* genes amplified from DNA extracted from the environment into the pGEMT-Easy vector (Promega) and using this as template DNA. The detection limit of the qPCR and RT-qPCR assays were 20 copies per 20 µl reaction.

For each environmental sample, copy numbers of the *dsyB*, *mmtN* and 16S rRNA genes/transcripts in the technical and biological triplicates were averaged and manually detected outliers were excluded from further analysis. In order to adjust for the differing copy number of 16S rRNA genes within prokaryotes, the copy numbers were normalised by dividing by 3.61, the average copy number in prokaryotes[46]. This was used to estimate a predicted percentage of *dsyB/mmtN*-containing bacteria. Statistical analysis was performed using Student's two-tailed *t*-tests in Microsoft Excel.

Analysis of public marine metagenomes and metatranscriptomes for MmtN

Hidden Markov Model (HMM)-based searches for MmtN homologues in metagenome and metatranscriptome datasets were performed as described in Curson *et al.*[16] using HMMER

tools (version 3.1, http://hmmer.janelia.org/)[47]. The MmtN protein sequences used as training sequences to create a HMM profile are listed in Supplementary Table 14. HMM searches were performed on OM-RGC database assemblies with an E value cut-off of $1e^{-30}$, and on selected *Tara* Oceans metatranscriptome databases (Supplementary Table 11) with an E value cut-off of $1e^{-5}$. Each potential MmtN sequence was manually curated using BLASTP analysis against the RefSeq database and discounted as a true MmtN sequence if the top hits were not to a recognised MmtN. The unique hits to MmtN in the metagenomes were normalised to the number of RecA sequences returned, giving an estimated percentage of *mmtN*-containing bacteria compared to *dsyB* and other genes involved in sulfur metabolism. For the metatranscriptomes, unique hits were normalised to gene length against the shortest gene, *dddK*.

Phylogenetic analysis of MmtN protein

MmtN amino acid sequences were aligned in MAFFT[48,49] v7 using default settings, then visually checked. Model selection and phylogeny construction were carried out using IQ-TREE v1.5.3[50], implemented in the W-IQ-TREE web interface[51]. The best supported model was LG+G4, and this model was used to build a phylogeny, with 1,000 ultrafast bootstrap replicates[52] used to assess node support. The tree was rooted using the MMT-like sequence from *A. thaliana*, and was formatted using the ggtree[53] package in R[54].

16S rRNA gene amplicon sequencing

16S rRNA gene amplicon sequencing of Stiffkey saltmarsh sediment samples was performed on at least three biological replicates from each condition by MR DNA (Shallowater, TX, USA), as described in Carrión *et al.*[41]. Sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity and taxonomy was assigned by BLASTn using a

curated NCBI and RDP database. Taxon-assigned data at the genus level for different samples across multiple runs were converted to count tables and joined in QIIME v1.9[55]. Samples were analysed at the genus level according to treatment group (Time 0, Control or Enriched). Data were normalised using total sum normalisation to convert raw counts to relative abundances.

The 50 most abundant genera were visualised in a bubble plot using R package ggplot2 v2.2.1[56]. Taxa with less than 0.01% mean relative abundance across all samples were removed, yielding a total of 330 genera after exclusion of 491 of the original 821 genera. Kruskal-Wallis rank sum test was used to assess if a significant difference existed at least once across treatment groups, then pairwise comparisons were made between treatment groups using Dunn's test with 'BH' *p*-value correction for multiple pairwise comparisons (Supplementary Table 6). Relative abundances of genera of interest were visualised in box plots using the R packages ggpubr v2.0[57], ggplot2 v2.2.1, and cowplot v0.9.2[58] (Supplementary Fig. 10).

Rarefaction curves were created using the R package vegan v2.4-6[59] to assess the sampling depth with average number of species (richness) plotted against number of reads sampled (Supplementary Fig. 3). The number of genera were plotted as a function of an even rarefied sampling depth of 36,066 sequence counts per sample. Data were normalised using total sum normalisation to convert raw counts to relative sequence abundances. Differences between DMSP-producing genera across treatment groups and DMSP gene categories were assessed for normality using the Shapiro-Wilks test, followed by analysis of variance, and Tukey multiple comparison of means test with a 95% confidence interval in the statistical package R[54].

dsyB diversity

To the study the diversity of the *dsyB* gene in environmental samples, extracted DNA was subjected to amplification with *dsyB* degenerate primers (dsyB_deg1F and dsyB_deg2R) and subsequently sequenced by MrDNA (Shallowater, Texas, USA) using Illumina MiSeq technology. Sequences were then analysed with QIIME[55] (Macqiime,version 1.9.0) to map the reads to a reference database constructed from 113 ratified DsyB amino acid sequences, with a 55% identity cutoff. Analysis yielded a total of 78,779 quality-filtered sequences with an average of 7,878 reads per sample. The resultant OTU table was sorted using an ID-mapping file identifying the phylogeny for each sequence.

Metagenomic analysis of Stiffkey saltmarsh sediment samples

DNA extracted from three biological replicates of Stiffkey saltmarsh sediment samples at Time 0 and samples incubated for 14 days under control or enriched conditions were combined in equal proportions to perform metagenomic analysis. Library construction and sequencing was conducted by MrDNA (Shallowater, Texas, USA) using Illumina HiSeq technology, as described in Carrión *et al* [41]. Following library preparation, the final concentration of the library was measured using the Qubit® dsDNA HS Assay Kit (Life Technologies), and the average library fragment size was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies). For Time 0 samples the average size was 826 bp, 931 bp for Control samples and 1,364 bp for Enriched samples. The library was pooled in equimolar ratios (2 nM), and sequenced paired end for 300 cycles using the HiSeq 2500 system (Illumina). Reads were quality-filtered and trimmed using Trimmomatic[60], obtaining an average of 15,363,915 reads per sample with an average length of 151 bp. Metagenomes were then assembled using SPAdes[61] assembler with kmers 55 to 127 and assemblies were analysed using Quast[62]. N50 values were ~1 kb for all metagenomes assemblies.

The abundance of functional genes in unassembled metagenomes was determined by Profile HMM-based searches (see 'Analysis of public marine metagenomes and metatranscriptomes for MmtN') of selected ratified gene sequences (*dsyB, mmtN, DSYB, Alma1, ddd* genes) against the raw reads ($E \leq e^{-4}$). Peptide databases were created by translating merged reads above 20 amino acids in length using the translate function in Sean Eddy's squid package (http://eddylab.org/software.html), as in Curson *et al*.[16]. Only unique hits were counted. The number of unique hits was normalised to read number of the smallest sample and to gene length, and bacterial genes were also normalised to number of RecA hits.

Cultivation studies

Media and growth conditions for bacteria

Thalassospira profundimaris DSM17430, *Pseudobacteriovorax antillogorgiicola* DSM103413, *Roseovarius indicus* DSM26383, *Labrenzia aggregata* LZB033, *Pelagibaca bermudensis* HTCC2597, *Novosphingobium* sp. BW1 and the other bacteria isolated from Stiffkey were grown in YTSS[63] or Difco Marine Broth 2216 (BD Life Sciences) complete medium, or MBM[64] (marine basal medium, adjusted to salinity of 35 PSU) (10 mM mixed carbon source from a 1 M stock of 200 mM succinate, glucose, pyruvate, sucrose and glycerol, and 0.5 or 10 mM NH_4Cl as nitrogen source as indicated) at 30°C. *Streptomyces mobaraensis* DSM40847 was grown in GYM *Streptomyces* medium (4 g glucose, 4 g yeast extract, 10 g malt extract, 2 g calcium carbonate, 12 g agar per litre distilled water) at 25°C and *Nocardiopsis chromatogenes* DSM44844 was grown in MYM medium (4 g glucose, 4 g yeast extract, 10 g malt extract, 2 g calcium carbonate, 10 g NaCl, 12 g agar per litre distilled water) at 37°C. *Corallococcus coralloides* DSM2259, *Stigmatella aurantiaca* DSM17044 and *Myxococcus fulvus* DSM16525 were grown in VY/2 medium (DSMZ medium 9) at 30°C. Where indicated, the salinity of MBM was adjusted by altering the amount of sea salts

(Sigma-Aldrich) added, and nitrogen levels were altered by adjusting the amount of NH_4Cl added. Methylated sulfur compounds, namely DMSP pathway intermediates, were added to MBM in the *Novosphingobium* intermediate incubation experiment. *Escherichia coli* was grown in Luria-Bertani (LB)[65] complete medium at 37°C. *Rhizobium leguminosarum* was grown in tryptone yeast (TY)[66] complete medium or Y[66] minimal medium (with 10 mM succinate as carbon source and 10 mM NH_4Cl as nitrogen source) at 28°C. Where necessary, antibiotics were added to media at the following concentrations: streptomycin (400 μg ml^{-1}), kanamycin (20 μg ml^{-1}), spectinomycin (200 μg ml^{-1}), gentamicin (20 μg ml^{-1}), ampicillin (100 μg ml^{-1}), rifampicin (400 μg ml^{-1}). Strains used in this study are listed in Supplementary Table 15.

Isolation and characterisation of DMSP-producing bacteria

The 'Time 0' and 'Enriched' samples from Stiffkey sediment enrichment experiments, see above, were serially diluted and plated onto MBM minimal medium. Plates were incubated at 28ºC for 72 h. Over 100 single colonies with different morphologies were purified and tested for DMSP production. Isolates of interest were identified by 16S rRNA gene amplification (using 27F/1492R) and sequencing as in Carrión *et al*.[41]. Bacterial isolates or type strains were assayed for DMSP production after 48 h growth in MBM (salinity 35 PSU, 0.5 mM NH_4Cl) by alkaline lysis and GC headspace analysis, see 'Quantification of DMS/DMSP/SMM by GC'. Where indicated, strains were instead either grown in MB medium or cells were scraped from MB agar plates into MBM medium prior to DMSP assays by GC. Cellular protein content was determined using the Bradford method (BioRad). *dsyB* degenerate primers were used to screen isolates for the presence of the gene (see 'Degenerate primer design').

Genome sequencing of Stiffkey isolates

Genomic DNA from *Novosphingobium* sp. BW1, *Stappia* sp. BW2, *Rhodobacterales* bacterium sp. BW5, *Marinobacter* sp. BW6 and *Rhodobacter* sp. BW8 was sequenced by MicrobesNG (Birmingham, UK) using Illumina technology. Resultant reads were trimmed with Trimmomatic[60] and quality-assessed using in-house scripts combined with the following software: Samtools[67], BedTools[68] and bwa-mem[69]. Annotation was performed with RAST, the NMPDR, SEED-based, prokaryotic genome annotation service (http://rast.nmpdr.org)[70], using the genome of the closest related strain as a reference.

DMSP production by cell lysates

For *Novosphingobium* cell lysate experiments, cultures were grown overnight in 5 ml YTSS medium, harvested by centrifugation at 20,000 *g* on a benchtop centrifuge for 5 mins and resuspended in 1 ml 50 mM Tris-HCl buffer (pH 7.5). Samples were sonicated to lyse the cells, then centrifuged at 20,000 *g* for 5 mins to pellet debris, and the lysate was removed. This lysate was dialysed to remove any pre-existing metabolites, using dialysis tubing (3,500 Da molecular weight cut-off, SpectrumLabs) in 2 l of dialysis buffer (20 mM HEPES, 150 mM NaCl, pH 7.5) at 4°C overnight. From this lysate 2 x 200 µl was mixed with either 1 mM SAM, 1 mM L-Met, or both, and then incubated for 30 min at room temperature. After incubation, assays were immediately transferred to 2 ml gas-tight GC vials, mixed with 100 µl 10 M NaOH and MMT activity was measured by GC, alongside heat-killed and buffer only controls (see 'Quantification of DMS/DMSP/SMM by gas chromatography').

In order to detect Met, SMM and DMSP-amine in *Novosphingobium* cell extracts, cultures were inoculated to a 5 ml YTSS starter culture and grown for 20 hours at 30°C. The starter culture was then centrifuged at 5,000 *g* for 1 min and resuspended in MBM medium twice. Washed *Novosphingobium* cells were then used to inoculate 1:100 to 100 ml MBM medium (35 PSU, 0.5 mM NH_4Cl, 10 mM mixed carbon source, 0.5 mM Met) and incubated at 30°C

for 20 hours. The entire 100 ml culture was then centrifuged at 5,000 *g* for 10 minutes and the cells were resuspended in 1 ml extraction buffer (50 mM potassium phosphate, 5 mM dithiothreitrol, 1 mM Na_2EDTA, 0.1 mM pyridoxal phosphate, 5 mM L-ascorbic acid, pH 7.2). Cells were sonicated (6 x 15 s) on ice using a Markson GE50 Ultrasonic Processor set to an output of 70. Sonicated *Novosphingobium* cells were centrifuged at 20,000 *g* on a benchtop centrifuge for 5 minutes and the supernatant was retained as cell extracts. Cell extracts were then analysed for Met, SMM and DMSP-amine as described in 'Quantification of Met, DMSP and SMM by HPLC' after 50- and 100-fold dilutions (n=2).

Seawater incubation experiments

Triplicate bacterial strains were grown overnight to stationary phase in MBM (for *P. bermudensis* and *Novosphingobium* sp. BW1) or YTSS (*L. aggregata* wild type and *dsyB*$^-$ strains, Supplementary Table 15). The cultures were harvested and washed three times with 0.2 µm filter-sterilised surface seawater (collected from Yarmouth estuary, latitude 52.6525, longitude 1.7336, September 2016 {0.07 ±0.001 nmol DMSP} for *L. aggregata* work or from Zhanqiao Pier, Qingdao, January 2018 {0.26 ±0.03 nmol DMSP }, for *P. bermudensis* and *Novosphingobium* sp. BW1 work). The resuspended cultures were adjusted to an OD_{600} of 0.4 and diluted 1:100 into 20 ml filter-sterilised seawater (T0), followed by incubation at 25°C for 21 h (T1) and 43 h (T2, not done for *L. aggregata*). From the T0, T1 and T2 samples, bacterial cells were harvested by centrifugation at 5,000 *g* for 5 mins and cell-free supernatants collected. The cell pellet was resuspended in 5 ml Tris-HCl buffer (50 mM, pH 7.5) and 500 µl 10 M NaOH was added (to chemically lyse the DMSP) to 2 ml of resuspended cells and cell-free supernatants in gas-tight vials and incubated in the dark overnight. Generated DMS was processed by a modified purge and trap method described by

689 Zhang *et al.*[30] and measured by GC, as above. There were no significant changes in DMSP
690 content in seawater only controls.

691 ***In vivo* and *in vitro* genetic manipulations**

692 Plasmids were transferred to *E. coli* by transformation, or to *R. leguminosarum* J391 or *T.*
693 *profundimaris* DSM17430 by conjugation in a triparental mating, using the helper plasmid
694 pRK2013[71]. Restriction enzyme reactions and ligations for cloning were done using Roche
695 enzymes according to the manufacturer's instructions. Standard PCR reactions were
696 performed using 2 x MyFi mastermix (Bioline). PCR products for sequencing or cloning
697 were purified using a Roche High Pure PCR purification kit. The oligonucleotide primers
698 used for molecular cloning were synthesised by Eurofins Genomics and are detailed in
699 Supplementary Table 17. Plasmids and PCR products were sequenced by Eurofins Genomics.
700 The amplified PCR products were then cloned into pLMB509, a vector used for expression in
701 *T. profundimaris*, or pET21a, an IPTG-inducible plasmid for the expression of genes in *E.*
702 *coli*, using *Nde*I and *Bam*HI or *Eco*RI restriction enzymes. All plasmid clones are described
703 in Supplementary Table 16.

704 **Library construction and cosmid screening of *Novosphingobium* sp. BW1**

705 A genomic library of *Novosphingobium* sp. BW1 was constructed essentially as described in
706 Carrión *et al.*[72]. *Novosphingobium* genomic DNA was extracted and partially digested with
707 *Eco*RI, ligated into the wide host-range cosmid vector pLAFR3 and transfected into *E. coli*
708 strain 803, to construct a library with an estimated 90,000 clones. The clones were transferred
709 *en masse* to *R. leguminosarum* J391 by conjugation. A total of 750 transconjugants were
710 picked to MBM medium containing 0.5 mM L-Met and screened by GC (see section
711 'Quantification of DMS/DMSP/SMM by GC') for those containing SMM (as a result of

conferred MMT activity). Two clones were identified that conferred MMT activity to *R. leguminosarum* J391, clone pBIO2279 and pBIO2280 (Supplementary Tables 8 and 16).

Identification of *mmtN*

The inserts in pBIO2279 and pBIO2280 were determined by sequencing their termini and aligning the sequence to the annotated genome sequences of *Novosphingobium* sp. MBES04 and BW1. Where the two fragments overlapped, the annotated genes were analysed by BLAST for candidate methyltransferase genes. The *mmtN* gene located was subcloned into pET21a and was shown to confer MMT activity to *E. coli*, as detailed above (Supplementary Table 8).

Identification of MmtN/MMT proteins in databases

BLAST searches[72] to identify homologues of the *Novosphingobium* sp. BW1 MmtN protein were performed using BLASTP at NCBI or JGI, as in Curson *et al*[16]. Representative strains containing MmtN homologues (E values $\leq 1e^{-50}$) were obtained, shown to produce DMSP and/or their *mmtN* genes were cloned and shown to confer MMT activity (as above). Thus, an E value of $\leq 1e^{-5}$ to a functional MmtN protein (Supplementary Table 14) was used as the cut-off to predict MMT functionality. Bacterial sequences with significant similarity to the larger plant-like MMT enzymes were identified using BLASTP, using the *Zea mays* MMT protein (NCBI accession: NP_001104941) as the query. Representative strains containing these MMT-like enzymes were obtained and assayed for DMSP production, as above.

Methionine *S*-methyltransferase (MMT) assays

To measure MMT activity from pET21a clones expressing the *mmtN* gene in *E. coli* BL21 (Supplementary Table 16), cultures were grown (in triplicate) overnight in LB medium, and

diluted 1:100 into 5 ml LB and incubated for 2 h at 37°C. This was then induced with a final concentration of 0.2 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) (Sigma-Aldrich) and incubated at 30°C overnight. For each culture, L-Met (Sigma-Aldrich) was added to 1 ml of culture (final concentration 0.5 mM) and incubated for 8 h at 30°C before assaying for SMM production by GC (see 'Quantification of DMS/DMSP/SMM by gas chromatography'). Protein concentrations were determined using the Bradford method (BioRad). *E. coli* BL21 containing the empty pET21a vector was used as a control.

MmtN protein purification and enzyme characterisation

Cultures of *E. coli* BL21 containing pBIO21N1 were grown in LB medium at 37°C, to an OD_{600} of 0.6–0.8, then induced at 20°C for 16 h with 0.3 mM IPTG. The MmtN protein was purified with Ni^{2+}-NTA resin (QIAGEN, Germany), and then fractionated using gel filtration buffer (10 mM Tris-HCl and 100 mM NaCl, [pH 8.0]) on a Superdex-200 column (GE Healthcare). MmtN purification was carried out at 4°C. For the Ni^{2+}-NTA resin purification, wash buffer (50 mM Tris-HCl, 250 mM NaCl and 20 mM imidazole, [pH 8.0]) was used to remove protein impurities, followed by the elution buffer (50 mM Tris-HCl, 250 mM NaCl and 250 mM imidazole, [pH 8.0]) to elute the purified protein from the column. MmtN enzyme activity was measured by monitoring the production of SAH (S-adenosyl homocysteine) produced by the demethylation of SAM, detected by HPLC through its UV absorbance under 260 nm. The standards for SAM and SAH were purchased from New England Biolabs, and Sigma-Aldrich respectively. During the reaction, the SAM was added in excess, and a standard curve of SAH was generated from a 1 mM stock that was diluted to concentrations in a range of 0-50 μM. Based on the standard curve, the peak area of SAH on HPLC was converted to SAH concentrations. Several different detection conditions were trialled, varying UV lengths and different phases to establish the following method for SAH

detection: SAH was measured by HPLC (Ultimate 3000, Dionex, America) on a SunFire C18 column (Waters, America) with a linear gradient of 1–20% acetonitrile in 50 mM ammonium acetate (pH 5.5) over 24 min at 260 nm.

LC-MS was used to confirm that SMM is produced when the pure MmtN enzyme *S*-methylates Met, using SAM as the methyl donor (Supplementary Fig. 13a). Optimal MmtN activity was determined by testing temperature and pH conditions, and comparing enzyme activity, with the highest activity defining 100% activity, and other tested conditions described as relative to it. The reaction mixtures were incubated at temperature intervals of 10°C, from 0°C to 60°C, for 30 min. For optimal pH levels, MmtN activity was examined using Britton–Robinson buffer (40 mM H_3BO_3, 40 mM H_3PO_4 and 40 mM CH_3COOH), at pH values between pH 5.0 and pH 10.0. Optimum conditions were pH 8.0, 30°C. In each of these assays, MmtN protein was used at the concentration indicated. Kinetic parameters (K_M) were determined by non-linear analysis, based on the initial rates and determined using 3.34 μM MmtN and 0.1-4 mM SAM, or 0.1-6 mM L-Met. The reaction mixture was incubated at 30°C for 30 min before detection. The enzyme activities were linear with respect to incubation time and enzyme concentration. Origin 8.5 was used to calculate K_M.

Gene mutagenesis in *T. profundimaris* DSM17430

Novosphingobium sp. BW1 was resistant to many antibiotics so *T. profundimaris* DSM17430 was used for gene knock-out experiments. Primers were designed (Supplementary Table 17) to amplify fragments internal to the *T. profundimaris* DSM17430 *mmtN* gene (WP_008888945, TH2_03115) and a closely linked aminotransferase (TH2_03140), which were cloned into pBIO1879[74], a derivative of the suicide vector pK19mob[75]. The resulting clones (Supplementary Table 16) were transferred into a spontaneous rifamipicin-resistant derivative (strain J595) of *T. profundimaris* DSM17430 by tri-parental conjugation using the

helper strain *E. coli* pRK2013. The *T. profundimaris* gene insertional mutants J596 (*mmtN*$^-$ mutant) and J597 (aminotransferase mutant) were isolated on YTSS agar containing rifampicin (J595), kanamycin (pBIO1879) and spectinomycin (pBIO1879). All mutants were ratified by PCR and checked for their ability to synthesise DMSP.

To confirm that the *mmtN* mutation in *mmtN*$^-$ mutant strain J596 (Supplementary Table 14) was responsible for the loss of DMSP production phenotype, cloned *Novosphingobium mmtN* (pBIO509N) was mobilised into J596 through tri-parental crossing.

Phenotyping of *T. profundimaris* mutant

Where MBM was used as the minimal medium for the following experiments, this medium lacked any methylated sulfur DMSP pathway intermediates. To identify potential phenotypes for the mutations in *mmtN*, the J595 (wild type) and J596 (*mmtN*$^-$) strains were grown with varying levels of salt and nitrogen, or under different environmental conditions, as in Curson *et al.*[8]. Strains were tested against 35 and 50 PSU for salt tolerance and 10, 0.5 or 0.1 mM NH_4Cl for different nitrogen levels, and growth was measured by OD_{600}. Tolerance to freezing was also tested, as in Curson *et al.*[8]. Competition experiments were performed in which cultures of the wild type and mutant strains were grown to stationary phase in 35 PSU MBM (10 mM NH_4Cl), OD_{600} adjusted, mixed in equal parts (500 µl of both) and subjected to high salinity (50 PSU) and reduced nitrogen (0.5 mM). Prior to and after perturbation, aliquots of the mix were serially diluted and plated on MB agar. Single colonies were tested for kanamycin/spectinomycin resistance (mutant selection) to distinguish the wild type from the mutant strain. All the above experiments used three biological replicates for each condition.

Data Availability

The 16S rRNA gene amplicon sequencing, metagenomic data and whole genome sequences generated in this study are publicly available at NCBI single read archive (BioProject PRJNA522699).

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Author contributions

J.D.T. wrote the paper, designed all experiments and did experiments; B.T.W. wrote the paper, designed all experiments and did/contributed to all experiments and prepared figures/tables; K.C. did experiments (genomic library screening, mutant complementation and characterised MMT^+ bacteria); A.B.M. did experiments (LC-MS work); A.R.J.C. did

experiments (genomic library construction, MMT assays, mutant construction, rate experiments); Y.Z. did experiments (qPCR, degenerate primer design, sampling and DMSP quantification in Mariana Trench); J.L. and J.L. did experiments (seawater incubations, qPCR, sediment sampling, purge-trap analysis, DNA/RNA purification from water); S.N-P., M.P. and C.Y.L. designed and did experiments (MmtN protein characterisation); P.P.L.R. did experiments (DMSP quantification in sediment, isolation and characterisation of eukaryotic species); L.G.S. wrote the paper and did experiments (evolutionary analysis of MmtN sequences and phylogenetic tree construction); C.A.B. devised experiments for measuring DMSP pathway intermediates in sediment and cell lysate by HPLC, carried out LC-MS experiments and discussed results; B.W.M. did experiments (16S rRNA amplicon sequencing analysis) and prepared figures; B.P. did experiments (cell lysate assays); J.P. did experiments (degenerate primer design, sediment sampling, bioinformatics analysis of metagenomic sequencing); O.C., X-H.Z., Y-Z. Z, J.C.M. designed experiments and discussed results.

Additional information

Supplementary information is available for paper. Reprints and permissions information is available at www.nature.com/reprints.

Competing interests

The authors declare no competing financial interests.

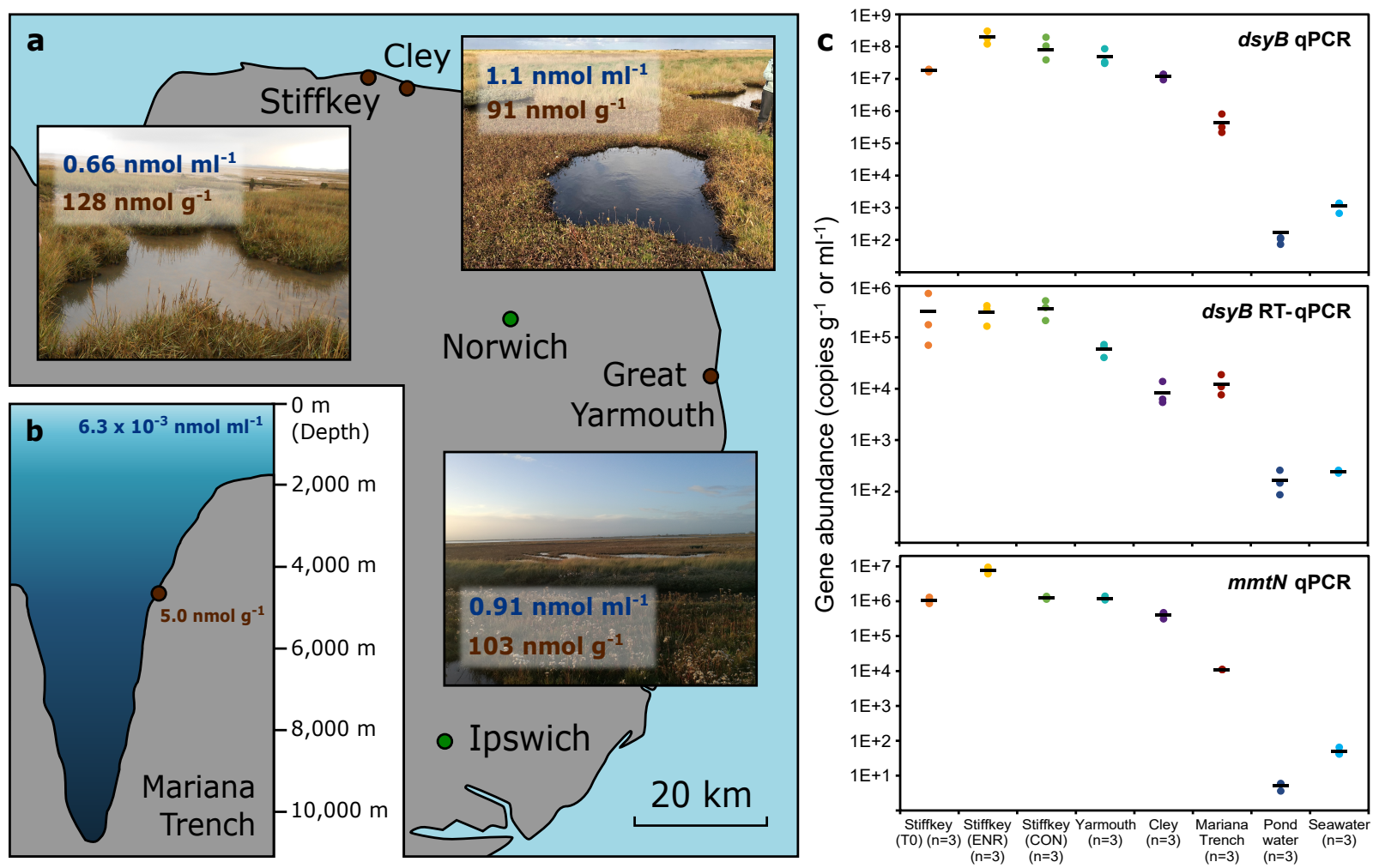
Figure 1. DMSP synthesis in tested marine sediments. (a and b) The mean standing stock concentration of DMSP in surface sediment (brown) and the overlying water (blue) from two saltmarshes Stiffkey and Cley, from an estuary (Yarmouth), and from the surface seawater (blue) and 4,500 m deep surface sediment (red) from the Challenger Deep of the Mariana trench (n=3 biologically independent samples). (c) qPCR work done on DNA (qPCR) and on mRNA (RT-qPCR) isolated from Stiffkey saltmarsh natural sediment (T0) and incubated sediment samples (control {CON}and enriched {ENR}for DMSP production); Yarmouth estuary sediment, Cley saltmarsh sediment and Mariana Trench 4,500 m deep surface sediment samples; and on Stiffkey saltmarsh pond water and coastal Great Yarmouth seawater samples. qPCR was done using degenerate primers designed to the DMSP synthesis genes *dsyB* and *mmtN* (n=2 and n=3 independent samples, the black line represents the mean value).

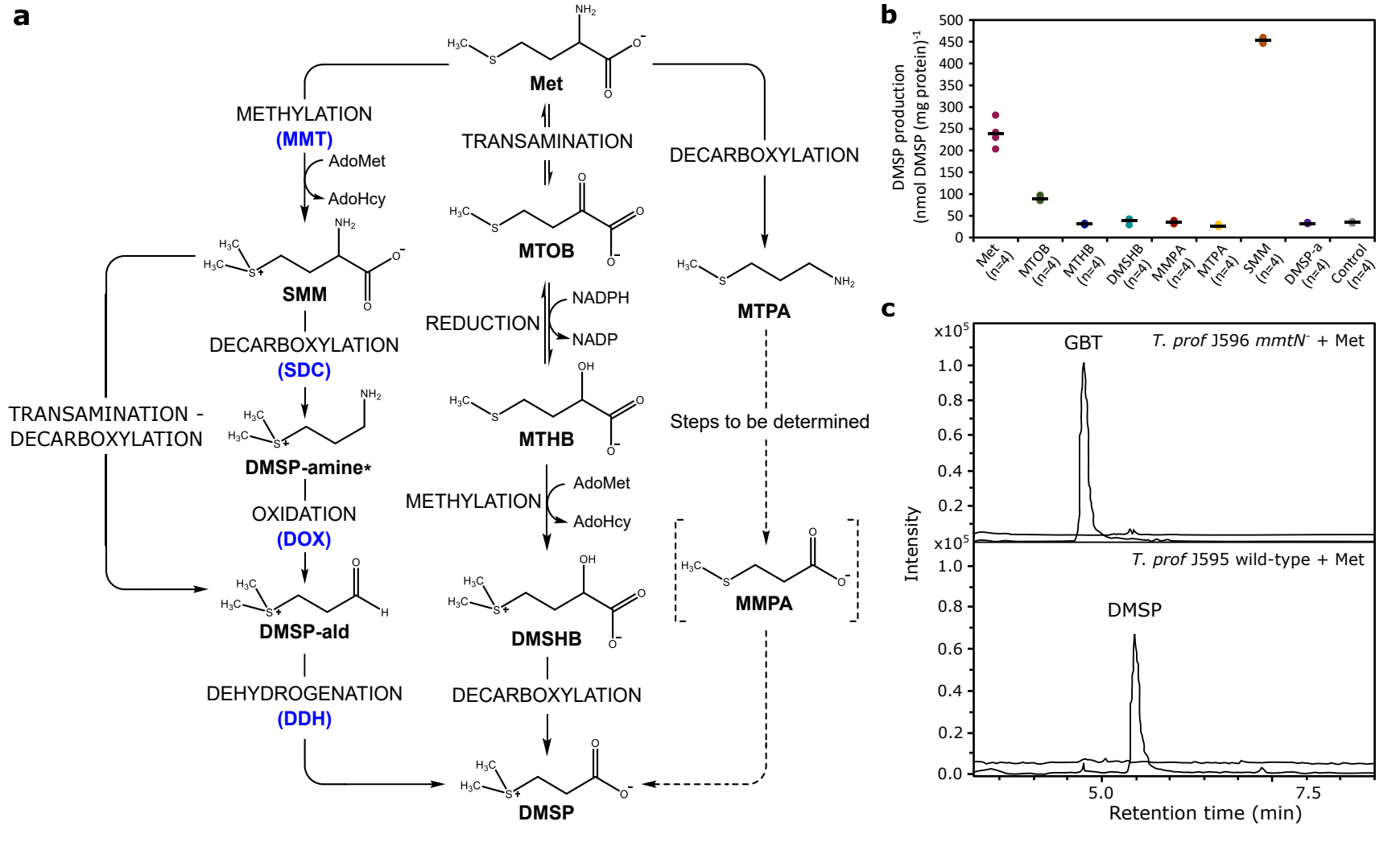
Table 1. DMSP synthesis rates and DMS production after 24 h using ^{3}H-Methionine, determined from saltmarsh and estuary samples from North Norfolk.

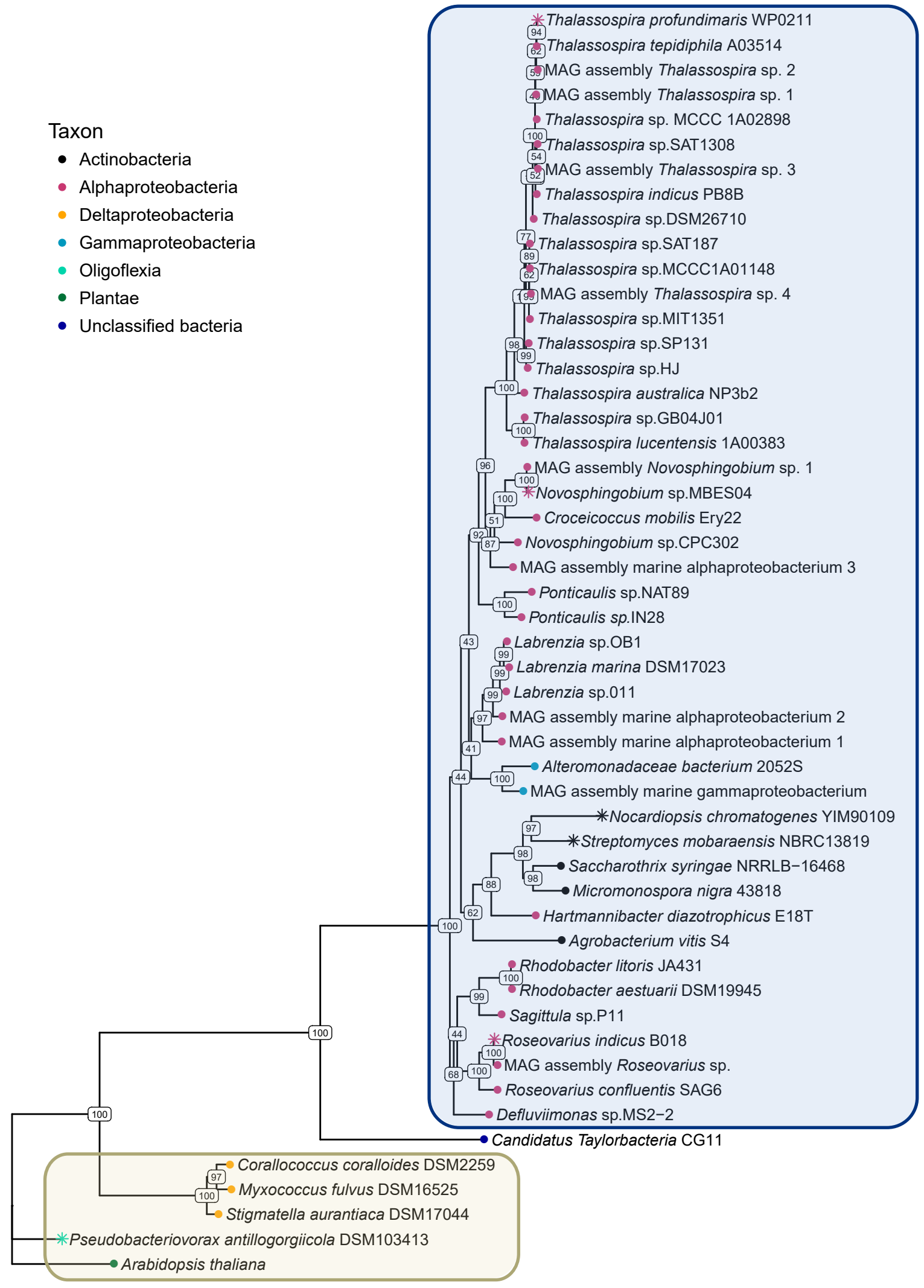
Sampling site	DMSP synthesis rate (fmol DMSP g^{-1} min^{-1} or ml^{-1} min^{-1})	pmol DMSP m^{-2} h^{-1}	pmol DMS g^{-1} captured after 24h
Stiffkey			
Sediment	263	158	1.89
Water	0.57		
Yarmouth			
Sediment	135	81.5	0.04
Water	2.27		
Cley			
Sediment	145	85.8	1.89
Water	1.13		

Figure 2. DMSP biosynthesis pathways and bacterial DMSP production. (a) Predicted pathways for DMSP biosynthesis edited from Curson *et al*[8] in higher plants and bacteria containing *mmtN* (*Spartina,**SMM is converted to DMSP-aldehyde (DMSP-ald) via an unconfirmed process in *Wollastonia*) (left); macroalgae (*Ulva*, *Enteromorpha*), diatoms (*Thalassiosira*, *Melosira*), prymnesiophytes (*Emiliania*), prasinophytes (*Tetraselmis*) (centre), algae that contain *DSYB* and bacteria that contain *dsyB*; and the dinoflagellate *Crypthecodinium* (right). The dotted line represents a suggested but as yet unconfirmed pathway. Enzymes involved in the *Spartina* pathway are in blue (MMT, methionine methyltransferase; SDC, *S*-methylmethionine decarboxylase; DOX, DMSP-amine oxidase; DDH, DMSP-aldehyde dehydrogenase). Abbreviations: SMM, *S*-methylmethionine, Met, methionine; MTOB, 4-methylthio-2-oxobutyrate; MTHB, 4-methylthio-2-hydroxybutyrate; DMSHB, 4-dimethylsulfonio-2-hydroxybutyrate, MTPA, 3-methylthiopropylamine, MMPA, methylmercaptopropionate. (b) *Novosphingobium* sp. BW1 DMSP production with or without (control) pathway intermediates (0.5 mM) in MBM minimal medium (10 mM succinate as carbon source, 10 mM NH_4Cl as nitrogen source). (n=4 independent samples, the black line represents the mean value). Student's two-tailed *t*-test ($P<0.05$): Met ($p=0.001$), SMM ($p=0.000001$) and MTOB ($p=0.0002$) were all significantly different to no addition (Control). (c) LC-MS chromatograms for DMSP (m/z 135) and GBT (glycine betaine) (m/z 118) in *Thalassospira profundimaris* J595 wild type (contains *mmtN*), compared to the J596 *mmtN*¯ mutant. These experiments were repeated twice with similar results.

Figure 3. Maximum-likelihood phylogenetic tree of MmtN proteins. Species are colour-coded according to taxonomic class as shown in the key, with proteins shown to be functional marked with an asterisk. Bootstrap support for nodes is marked. Bacterial MmtN proteins are boxed in blue and the larger MMT proteins are boxed in cream. Based on 47 protein sequences.


a
Cley
Stiffkey
1.1 nmol ml-1
91 nmol g-1
0.66 nmol ml-1
128 nmol g-1
Norwich
Great Yarmouth
0.91 nmol ml-1
103 nmol g-1
Ipswich
20 km
b
6.3 x 10-3 nmol ml-1
0 m (Depth)
2,000 m
4,000 m
6,000 m
8,000 m
10,000 m
5.0 nmol g-1
Mariana Trench
c
dsyB qPCR
dsyB RT-qPCR
mmtN qPCR
Gene abundance (copies g-1 or ml-1)
Stiffkey (T0) (n=3)
Stiffkey (ENR) (n=3)
Stiffkey (CON) (n=3)
Yarmouth (n=3)
Cley (n=3)
Mariana Trench (n=3)
Pond water (n=3)
Seawater (n=3)


a
Met
METHYLATION
(MMT)
AdoMet
AdoHcy
SMM
DECARBOXYLATION
(SDC)
DMSP-amine*
OXIDATION
(DOX)
DMSP-ald
DEHYDROGENATION
(DDH)
TRANSAMINATION -
DECARBOXYLATION
TRANSAMINATION
MTOB
REDUCTION
NADPH
NADP
MTHB
METHYLATION
AdoMet
AdoHcy
DMSHB
DECARBOXYLATION
DECARBOXYLATION
MTPA
Steps to be determined
MMPA
DMSP
b
DMSP production
(nmol DMSP (mg protein)-1
Met (n=4)
MTOB (n=4)
MTHB (n=4)
DMSHB (n=4)
MMPA (n=4)
MTPA (n=4)
SMM (n=4)
DMSP-a (n=4)
Control (n=4)
c
GBT
T. prof J596 mmtN- + Met
T. prof J595 wild-type + Met
DMSP
Intensity
Retention time (min)


Taxon
Actinobacteria
Alphaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
Oligoflexia
Plantae
Unclassified bacteria
Thalassospira profundimaris WP0211
Thalassospira tepidiphila A03514
MAG assembly Thalassospira sp. 2
MAG assembly Thalassospira sp. 1
Thalassospira sp. MCCC 1A02898
Thalassospira sp.SAT1308
MAG assembly Thalassospira sp. 3
Thalassospira indicus PB8B
Thalassospira sp.DSM26710
Thalassospira sp.SAT187
Thalassospira sp.MCCC1A01148
MAG assembly Thalassospira sp. 4
Thalassospira sp.MIT1351
Thalassospira sp.SP131
Thalassospira sp.HJ
Thalassospira australica NP3b2
Thalassospira sp.GB04J01
Thalassospira lucentensis 1A00383
MAG assembly Novosphingobium sp. 1
Novosphingobium sp.MBES04
Croceicoccus mobilis Ery22
Novosphingobium sp.CPC302
MAG assembly marine alphaproteobacterium 3
Ponticaulis sp.NAT89
Ponticaulis sp.IN28
Labrenzia sp.OB1
Labrenzia marina DSM17023
Labrenzia sp.011
MAG assembly marine alphaproteobacterium 2
MAG assembly marine alphaproteobacterium 1
Alteromonadaceae bacterium 2052S
MAG assembly marine gammaproteobacterium
Nocardiopsis chromatogenes YIM90109
Streptomyces mobaraensis NBRC13819
Saccharothrix syringae NRRLB−16468
Micromonospora nigra 43818
Hartmannibacter diazotrophicus E18T
Agrobacterium vitis S4
Rhodobacter litoris JA431
Rhodobacter aestuarii DSM19945
Sagittula sp.P11
Roseovarius indicus B018
MAG assembly Roseovarius sp.
Roseovarius confluentis SAG6
Defluviimonas sp.MS2−2
Candidatus Taylorbacteria CG11
Corallococcus coralloides DSM2259
Myxococcus fulvus DSM16525
Stigmatella aurantiaca DSM17044
Pseudobacteriovorax antillogorgiicola DSM103413
Arabidopsis thaliana